Synopsis

Pharmacognostic Studies of Some Ethnomedicinal Plants Being Used for Hepatoprotective Activity in Kolhan Division of Jharkhand

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STATE OF ART

There has been a relationship between indigenous people and the plants since ancient era. People have sought to cure illness through nature. Theophrastus, a Greek scientist, founded the *School of Medicinal Plants*. After then, Dioscorides, a physician and surgeon (75-45 BC), wrote an encyclopaedia called *De Materia Medica,* in which he described 600 therapeutic medicinal plants as a series of scientific studies on medicinal plants [1][2][3]. Two textbooks Charaka Samhita by ‘‘Charaka’’ and Sushruta Samhita by ‘‘Sushruta’’ were assembled which deals with the etiology, symptomatology, pathology, prognosis, and medical management of disease and various surgical instruments and procedures [4][5]. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine [6].

India has rich knowledge on traditional practices to cure various ailments from the past days. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy are Indian traditional medical systems since ancient period [7]. The use of herbal medicine becomes popular due to toxicity and side effects of allopathic medicines. This led to sudden increase in the number of herbal drug manufactures [8].

Jharkhand (“The land of forest”) is the 28th state of India. Kolhan division is one of the five division of Jharkhand. These comprises three districts that is West Singhbhum, Seraikela-Kharsawan and East Singhbhum. These districts are inhabited by mostly tribal population and they were known as Hos or Larka or Kols. Their livelihood generally depends on agriculture, selling of vegetables/ crops in weekly markets, working as labours, etc. The diverse forests of Jharkhand house several wild weeds leafy plants that have been used from centuries by the indigenous communities of the region. The indigenous knowledge of these ethnomedicinal plants has been passed down through generations, and they continue to play a vital role in the primary healthcare system of many rural communities in Jharkhand [9].

‘Pharmacognosy’ word is derived from the Greek words *pharmakon* (drug) and *gnosis* (knowledge). It is defined as ‘study of physical, chemical, biological properties of drugs or drug substance of natural origin. It deals with the study of crude drugs obtained from the medicinal plants, animals or minerals constituents. Further, it deals with standardization, authentication, identification of the quality of natural products through various parameters such as organoleptic characters, macroscopic or microscopic characters, phytochemical, physicochemical analysis, etc [10]. The research in Pharmacognosy have changed from

traditional knowledge to modern technologies. It emphasizes and focus on research in identification of drugs, isolation of active components, investigation of biological activity [11].

These crude drugs have a very potential role in curing diseases. Since ancient times, various bioactive compounds have been discovered for e.g. atropin, ephedrine, morphine, caffeine, salicylic acid, digoxin, taxol, galantamine, vincristine, colchicine, etc. which played an important role in human life [12].

Hepatoprotective activity

Hepatoprotection means ability to protect the liver cells from any damage caused due to any oxidative stress or toxins or viral attack, etc through some hepatoprotective agents such as herbal drugs of plants [13].

Liver is the largest gland in the body. It has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as detoxification of metabolites, synthesis and production of proteins and other necessary digestive enzymes and breakdown/ emulsification of fats, secretion and storage, etc. The term liver is derived from Greek word 'hepat'; hence it is termed as hepatic organ. Till now there is no way to compensate absence of liver function in long term condition, liver dialysis can be done in short term basis in case of any diseased condition and liver transplant in case of liver failure [14].

Most of the hepatotoxic chemicals such as paracetamol, CCl_4 etc. damage liver cells by inducing lipid peroxidation or other oxidative damages. Several types of liver diseases are genetic or caused by viruses or other illness, or by toxins such as drugs/ alcohol. Some liver diseases are hepatitis, alcohol-associated liver diseases, non-alcoholic fatty liver diseases (NAFLD), liver cancer, jaundice, cirrhosis etc. Such diseased condition also results in damage of liver cells [15][16].

Wild leafy vegetables which treat several diseases such as cancer, diabetes, hypertension, anaemia, malaria, cough- cold, fever, constipation, etc are *Moringa oleifera* [26][27], *Tinospora cordifolia*, *Asparagus racemosus*, *Centella asiatica*, *Achyranthes aspera*, *Aerva lanata, Boerhaavia diffusa* etc. [17]. The state report about consumption of alcoholic beverage or traditional rice beer around the age group of 46 years was 6.16 % of 106 patients [18]. The community-based assessment checklist prepared by Jharkhand health department is under progress which report occurrence of liver diseases such as NAFLD, ALD, etc. in rural areas of Jharkhand [19]

Plants are repository of many important metabolites. Primary and secondary metabolites include compounds such as carbohydrates, proteins, vitamins, tannin, phenol,

alkaloids, coumarins, essential oils, saponin, etc. These metabolites play an important role in proper metabolic function of the cell, defense mechanism, growth, etc.

In the recent research proposal, various ethnomedicinal plants i.e. wild leafy edible plants will be explored which have the potential to prevent liver from any damage by interviewing Vaidyas or Herbal Practitioner or Ayurvedic Chikitshak in different districts of Kolhan division of Jharkhand. The secondary metabolites will be screened to confirm their hepatoprotective activity. Some of the ethnomedicinal leafy plants which have the ability to cure hepatic diseases on the basis of literature survey from reviewed research papers are as follows:

1. *Leucas aspera* (Willd.) Link

Leucas aspera (Family - Lamiaceae) is a wild edible weed plant of Jharkhand. It is commonly known as 'Thumbai' in India, 'Dronapushpi' in Sanskrit. It is an annual, branched, herb with a height of 15-60 cm quadrangular stem and branches. A small white sessile, axillary or terminal whorl of flower with bracts appear and fruit are nutlets, 2.5 mm long, oblong, brown, smooth, inner face angular and outer face rounded [20][21].

Various bioactive phenolics or alkaloids such as triterpenoids [22], oleanolic acid, ursolic acid and 3-sitosterol [23], reducing sugars (galactose), glucoside [24], diterpenes [25], etc have been reported.

The juice of the leaves is used as remedies for psoriasis, chronic skin eruptions and chronic rheumatism [26]. The flowers are given with honey to treat cough and cold in children. The leaves are applied to the bites of serpents, poisonous insects and scorpion sting. *L. aspera* leaves are also used as insecticides and mosquito repellent in rural areas [27]. The plant extract with honey is a good remedy for stomach pain and indigestion. It has anti-fungal and fungicidal property [28], anti-microbial [29], antinociceptive, antioxidant and cytotoxic properties [30] and hepatoprotective activity [31]. The leaves and stem extract also showed anti-hyperglycaemic activity in glucose-challenged Swiss albino mice [32].

2. *Chenopodium album* L.

Chenopodium album L. is a fast-growing annual, invasive weedy plant in the fields of wheat, barley, mustard, gram and other crops [33][34], belonging to the Chenopodiaceae family, which is widely distributed in hot sub-tropical and tropical climates, as well as temperate regions of the world.

The stem grows to a height of 4 inches to 6 feet, upright, branched, erect, grooved, striped with green to greyish and red in colour. Leaves are simple, opposite, glabrous with lower leaves resembles a goose's foot, wax-coated, unwettable; leaf margin and undersides are sometimes purplish. Flowers are radial, symmetrical, green and white, in small clusters on a

dense branched inflorescence, 10-40 cm long, contains shining black seeds and continuous along the stem. The seed remains enclosed by flower segments at maturity [35- 38].

The whole young plant has been reported as food and herbal medicine. *C. album* is known as *Bathua sag* (Hindi). Studies on various phytochemical constituents of *C. album* have indicated that the plant contains phytochemicals with various pharmacological effects, including antiviral, antifungal, antioxidant, anti-inflammatory, antiallergic and antiseptic activities [39-41]. The leaves further show hepatoprotective [42], anti-diarroheal [43], anti-bacterial [44], anticancer activity [45]. The chief secondary metabolites isolated from different parts of the plant included alkaloids, glycosides, non-polar lipid, ascorbic acid, phenols, lignins, flavonoids, saponins, β-carotene, catechin, gallocatechin, caffeic acid, p coumaric acid, ferulic acid, β-sitosterol, campesterol, xanthotoxin, stigmasterol, n-triacontanol, imperatorin, ecdysteroid[46][47], crytomeridiol[48], ntransferuloyl- 4-O-methyl dopamine , β-sitosterol, lupeol and 3 hydroxy-nonadecylhenicosanoate [49][50] .The infusion and decoction of leaves, roots and inflorescence had showed a positive response against helminths[51].

3. ***Hygrophila polysperma*** (Roxb.) T.Anderson.

The plant is semi-aquatic or amphibious herb native to Asia [52] belonging to family Acanthacea. The genus contains 80 species that occurs in tropics of the world [53]. In India, 9 species of *Hygrophila* have been reported. It is commonly known as 'Dwarf Hygro' in tropical and sub-tropical regions of India and Malaysia [54][55]. In Jharkhand, it is an edible leafy vegetable which grow as weeds and known as 'Muchari saag'.

H.polysperma has been used in ayurvedic medication for facial paralysis, stiff-neck, hemiplegia and noise in ears with headache [56]. The seeds have also been used for treating various diseases in India [57] and are reported in the medicinal plant lists of Indian states of West Bengal [58] and Karnataka [59]. Phytochemical screening of this weed is less reported.

They play ecological and functional roles in various aspects, such as assimilating excessive nutrients (e.g., nitrogen and phosphorus) in eutrophic waters [60][61] removing heavy metals and nanoparticles in wastewater [62-66]. It is an important bio-indicator for surfactant toxicity level [67] as well as for algae and Indian ferns in aquarium. Terpinene-4-ol phytochemical has been reported in leaves and seeds of the plant [68].

PROBLEMS TO BE INVESTIGATED

Kolhan division of Jharkhand is a house of variety of flora and fauna in its area. It is a division of tribal people also. Majority of the areas have uneducated people due to

economically weak status. In such a situation if a person is diagnosed with a dreadful liver disease, then it becomes difficult for them to treat it by various medical therapies (due to expensive in nature).

Liver, an important organ of the body, if fails to function, no compensation organ is available. Numerous liver diseases such as liver cancer (HCC), jaundice, hepatitis, alcohol-associated, non-alcohol associated liver diseases are prevalence in the Kolhan division of Jharkhand.

Eradication of problems facing liver diseases is the priority of this research. Ethnomedicinal edible plants will be investigated that has the property to keep the liver organ healthy.

OBJECTIVE OF THE INVESTIGATION & MOTIVATION

Objectives:

I. To collect, identify and authenticate ethnomedicinal leafy plants bearing hepatoprotective capability by Herbal Practitioners in the Kolhan Division of Jharkhand.
II. To study the physico-chemical properties of selected ethnomedicinal plants using standard protocol.
III. To study the qualitative and quantitative phytochemical analysis of the selected ethnomedicinal plants.
IV. To study the antioxidant activity of selected ethnomedicinal plants using standard protocol.

Motivation:

The Kolhan division of Jharkhand are inhabited by economically weaker people. Any kind of expensive treatment due to certain fatal diseases may affect their livelihood. Most of the population of Jharkhand and India are feeding unhealthy diets, alcohols, etc due to which the most important and crucial organ i.e. liver is affected. Thus, protection of liver organ is the priority of this research.

With the advent of technology, people are following an unhealthy lifestyle and are facing various health issues. Traditional practices and ethnomedicines, which have been used for centuries, have the ability to cure even dreadful diseases at their root. Therefore, such

traditional knowledge and practices should be followed so that people can live healthier, safer, and happier lives with minimal side effects.

HYPOTHESIS

The present investigation for PhD research will explore and authenticate the plants and also correlate efficiency of bioactive compound present in medicinal plant responsible for hepatoprotective activity.

RESEARCH DESIGN AND METHODOLOGY

I. **Material**:

Plant material: The plant sample selected for the proposed research work belongs to different families. The information has been gathered from reviews and literature from indexed journals, articles, etc. The collection and authentication of ethnomedicinal plant sample will be done under the supervision of experts and taxonomist.

II. Methodology

1. **Survey-** Interview of Vaidyas/ Herbal Practitioner/ elderly people/ indigenous people will be conducted in few blocks of Kolhan division of Jharkhand using questionnaire related to liver diseases.
2. **Physico-chemical analysis**: The physical and chemical properties of ethnomedicinal plants will be conducted using parameters such as colour, taste, percent of loss on drying, ash content, ash value, extractive values, presence of inorganic and organic elements will be done using standard protocol [69].
3. **Phytochemical screening and identification of bioactive compounds**: Standard protocol will be followed to screen the qualitative or quantitative estimation of various extracts of ethnomedicinal plant and presence of bioactive compounds using GC-MS analysis and correlate the bioactive compounds in treating the liver diseases [70][71].
4. **Antioxidant efficacy**: The antioxidant activity was determined by radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and cupric ion reducing ability and attempt will be made to correlate antioxidant efficacy in curing the diseases[72].
5. **Hepatoprotective activity by cytotoxicity and cell viability assay-** Toxic induced liver cell culture viability was assessed by MTT assay ((3- [4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) to confirm the hepatoprotective activity of plant

extract. MTT reduction assay is a colorimetric assay in which MTT is converted to purple formazan derivative by mitochondrial succinate dehydrogenase, which is present only in viable cells.The cells were treated with plant extract and/or the toxic agent. After then, medium was removed and the cells were incubated with MTT (0.5 mg/ml) for 2 hours. Then formazan crystals were dissolved with 200 μL/well of DMSO solvent. Absorbance was measured at 570 nm. Viability was defined as the ratio of the absorbance of treated cells to that of untreated control cells and is expressed as a percentage [73].

TOOLS TO BE USED IN COLLECTION OF DATA

a. **List of instruments: -** Spectrophotometer, Weighing Machine, Vortex, HPLC/GC-MS, Laminar Air Flow, Incubator, Atomic Absorption Spectrophotometer, pH meter, Conductivity meter, Colorimeter etc.
b. **Glasswares**: Petri-plate, Measuring cylinder, Conical flask, Beaker, Eppendorf tubes, Test tubes, etc.
c. **Chemicals:** DMSO, methanol, ethanol, chloroform, distilled water, DPPH, neocuproine, acetic acid, Tris-HCl, sodium chloride, ferric chloride, quercetin, ascorbic acid 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), and CCl4 (99.9%), Dulbecco's modified Eagle's medium advanced (DMEMA) with and without phenol red, foetal bovine serum, trypsin 0.25% (1×), penicillin G (100 IU/mL), streptomycin (100 μg/mL), and phosphate-buffered saline (PBS) etc.
d. **Cell culture**- Hep G2 human liver hepatoma cells will be procured from NCCS, Pune. If the cell line does not survive or show negative response then alternative cell will be selected as per the availability.

SIGNIFICANCE OF THE STUDY

Traditional knowledge has its own importance to cure any disease. Many ethnomedicinal plants have been recognized for their hepatoprotective properties, with many traditional healing systems utilizing them to support liver health and treat liver-related disorders. Numerous plant species possess bioactive secondary metabolites such as phenolics, terpenes, alkaloids, essential oils, flavonoids, etc have demonstrated the potential to protect the liver from damage induced by toxins, inflammation, and oxidative stress. These metabolites prevent liver from damage through activities such as antioxidant, anti-

inflammatory and anti-fibrotic effects thereby enhancing liver function and promoting regeneration of damaged hepatic tissues.

As treatment of liver disorders are very costly, in economically weaker section, use of herbal medicines will help them to cure any dangerous disease. Scientific studies support the efficacy of several ethnomedicinal plants in mitigating liver diseases, including hepatitis, cirrhosis, and fatty liver disease, by reducing hepatocellular injury, improving bile secretion, and inhibiting fibrogenesis. Consequently, these plants provide valuable avenues for the development of natural hepatoprotective agents and complementary therapeutic strategies in modern hepatology.

IT'S RELEVANCE TO THE PRESENT-DAY PROBLEMS AND NEED OF THE SOCIETY

As herbal plants have nutraceutical and pharmaceutical properties, some of the species can be consumed and cultivated. In recent decades ethno-medicinal plants are gaining popularity due to their nutritive value and possible potential therapeutic uses for health. Herbal plants are known to complement chemotherapy and radiation therapy by countering the side-effects of chemical drugs.

Thus, herbal plants can also be a good remedy for curing various diseases. From the present investigation, herbal plants efficacy for treating liver diseases will be authenticated, which will help the society and especially the poor section.

FUTURE PROSPECTIVE

Herbal plants products are set to create a revolution in therapeutic strategies in curbing various forms of diseases. Through this investigation authentication to usage and manufacture of medicine can be achieved. Many unknown ethnomedicinal plants need more investigation and exploration. Also, mechanism underlying biological action against various liver diseases will accelerate commercial production of pharmaceuticals for treatment. Furthermore, molecular characterization and gene sequencing of the selected plants could be ascertained.

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